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(71) Applicant *(for all designated States except US)*: PRINCETON UNIVERSITY [US/US]; New South Building, 5th floor, P.O. Box 36, Princeton, NJ 08544-0036 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: LEMISCHKA, Ihor [US/US]; 4 Firestone Court, Princeton, NJ 08540 (US). MOORE, Kateri [US/US]; 248 Hawthorne Street, Princeton, NJ 08540 (US).

(74) Agents: TAMIN, Michael, A. et al.; Klauber & Jackson, 411 Hackensack Avenue, Hackensack, NJ 07601 (US).

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(54) Title: GENES THAT REGULATE HEMATOPOIETIC BLOOD FORMING STEM CELLS AND USES THEREOF

(57) Abstract

The present invention provides an isolated nucleic acid derived from an isolated hematopoietic stem cell. The present invention additionally provides an isolated hematopoietic stem cell specific protein or a portion thereof encoded by the provided nucleic acid. In addition, the present invention provides a method for generating a stem cell/progenitor cell from a primitive hematopoietic cell. Also, the present invention further provides a method for identifying the presence in a sample of a compound that modulates hematopoietic stem cell activity. The present invention additionally provides a molecularly defined primitive hematopoietic stem cell. Finally, the present invention provides a method of ex vivo expansion of hematopoietic stem cells.

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GENES THAT REGULATE HEMATOPOIETIC BLOOD FORMING STEM CELLS AND USES THEREOF

GOVERNMENTAL SUPPORT

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Throughout this application, various publications are referenced by number. Full citations for these publications may be found listed at the end of the specification and preceding the Claims. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art. A Sequence Listing is provided.

FIELD OF THE INVENTION

The present invention relates to hematopoietic stem cells and the stem cell and support cell genes that support stem cell replication and differentiation.

BACKGROUND OF THE INVENTION

The adult hematopoietic system is organized as a hierarchy of cells with decreasing self-renewal and multilineage differentiation potential. This is accompanied by progressively larger numbers of more mature cells and an increasing tendency to be in active cell cycle (Lemischka, I.R., 1992; Morrison, S.J., et al. 1995). Collectively, the properties of this hierarchical system result in the balanced, lifelong production of at least eight distinct cell lineages. A population of stem cells establishes the entire hierarchy; therefore, in order to understand the fundamental mechanisms responsible for normal hematopoiesis it is ultimately necessary to understand the biology of the stem cells.

Most information concerning the biology of stem cells has been obtained from the mouse model. In this system, the most critical, characteristic property of the stem cell population has been defined; that is, its ability to reconstitute a normal blood system in a transplanted host. A number of variations on the basic transplantation assay have been

described (Harrison, D.E. 1980; Spangrude, G.J., et al. 1995). All of these systems, together with the appropriate donor vs. host or clonotypic markers have rigorously defined the most primitive stem cells and have provided a description of their developmental properties. Perhaps the most striking characteristics of this cell population come from retroviral "marking" studies (Leminschka, I.R. 1992). These studies clearly show that a single stem cell clone is both necessary and sufficient, not only to sustain lifelong, multilineage hematopoiesis in one primary recipient but in numerous secondary animals. This illustrates the remarkable proliferative potential of the stem cell and directly demonstrates stem cell self-renewal. A major advance in mouse stem cell biology was the development of strategies which facilitate the substantial enrichment of stem cell activity (Bauman, J.G., et al. 1988; Spangrude, G.J., et al. 1988; Jordan C.T. , et al 1990). Purification procedures enabled the first direct approaches to unravel the mechanisms responsible for the unique biological properties of the stem cell population. A key observation was that the phenotypically defined stem/progenitor cell population is heterogeneous with respect to in vivo functional properties (Fleming, W.G., et al 1993; Li, C.L. and Johnson G.R. 1992; Spangrude, G.J. and Johnson, G.R. 1990, Jones R., et al. 1990; Uchida, N., et al. 1993). In addition to the in vivo repopulating cells, other primitive progenitor cells are often contained in a purified population (Weilbaecher, K., 1991; Trevisan, M. and Iscove, N.N. 1995; Ogawa, M. 1993). These can be assayed in a variety of in vitro systems. Whether all of these in vivo and in vitro activities represent discrete cell subpopulations or whether there is a continuum of functional potential is still an unanswered question. Recent studies have suggested distinct physical properties for functionally different activities within the primitive population Morrison, S.J., and Weissman, I.L. 1994); Morrison, S.J. et al. 1997; Jones, R.M., el al. 1996). A second set of observations revealed an inverse correlation between a tendency for active cell cycling and primitive, uncommitted developmental potential in BM (Spangrude, G.J., and Johnson, G.R. 1990). In fetal liver a higher proportion of primitive stem cells is actively cycling (Fleming, W.H., et al. 1993). Moreover, it has been shown that fetal stem cells are more potent than adult stem cells in LTRA (Jordan, C.T., et al. 1995; Pawliuk, R., et al 1996). These are exciting observations because they suggest that rapid stem cell cycling can be compatible with the maintenance of primitive in vivo activity. Very recent studies suggest that the adult BM stem cell compartment may in fact be cycling at a very slow rate (Bradford, G.B., et al. 1997) . Clearly, stem cell cycle regulation is a critical

area for investigation. Stem cell purification has facilitated studies aimed at ex vivo maintenance or expansion of the most primitive, transplantable stem cell. Most culture systems strongly favor a differentiation process (Van der Sluijs, J.P., et al. 1993; Traycoff, C.M., et al. 1996; Peters, S.O., et al. 1995; Knobel, K.M., et al. 1994). However, several recent reports have been encouraging. It has been shown that colonies grown in defined cytokines can retain not only myeloid and erythroid but also lymphoid potentials (Ball, T.C., et al. 1995). Moreover, the short-term (2-3 weeks) maintenance of LTRA has been demonstrated in suspension cultures supported by IL6, IL11, together with ckit ligand (KL) or flk2/flt3 ligand (FL) (Yonemura, Y.H., et al. 1997). A recent report has shown that colonies initiated in cytokine-supplemented semisolid cultures retain LTRA (Trevisan, M., et al. 1996). The studies described herein have developed a stromal cell line supported system which quantitatively maintains LTRA for an extended (4-7 weeks) time (Moore, F.A., et al. 1997).

In the human system it is clearly not possible to do the same kind of extensive marking and transplantation assays. However, several xenograft model systems have been developed to assess the in vivo behavior of human stem cells (Traycoff, C., et al. 1994; Turner, C., et al. 1996; Cashman, J., et al. 1997) . Some of these experiments can be done quantitatively in limiting dilution (Bhatia, M., et al. 1997). A very recent study has demonstrated a common proviral integration site in granulocyte macrophages and T-cells derived from beige/nude/XID mice 7-11 months after engraftment with genetically transduced human stem cells (Nolta, J.A., et al. 1996) . This important study paves the way for precise in vivo clonal analyses. The largest amount of functional information about human stem/progenitor cells has been obtained in vitro using a wide range of stromal cell and cytokine supported culture systems. It is not possible herein to describe and properly accredit all of the important studies, however several advances deserve mention. The long-term culture-initiating cell (LTCIC) assay measures the in vitro production of colony forming cells (CFC) after periods of at least five weeks in culture (Sutherland, H.S., et al. 1989) . The cells producing these CFC derive from a population of cells which, at least to some extent, probably overlaps with the most primitive compartment. The maintenance and expansion of primitive functional abilities in this culture system has recently been documented (Petzer, A.L., et al. 1996) . A variation on this assay system, the extended LTCIC (ELTCIC) has been suggested to measure an even more primitive cell population in BM and CB (Hao, Q.L., et al. 1996) . A very exciting

prospect for the near future will be the integration of the various in vivo xenograft assays with the in vitro LTCIC and ELTCIC systems. Some very recent efforts have suggested that the NODSCID xenograft system and the LTCIC assay may measure distinct stem/progenitor subsets (Larochelle, A..J., et al. 1997). Clearly, much more work needs to be done, however, it may be anticipated that the ELTCIC system will provide the "bridge" in this continuum. Collectively, and including the various strictly cytokine-driven systems, the above studies illustrate the current possibility to accurately and quantitatively reveal the majority (if not all) functional entities in the human stem/progenitor cell hierarchy. The physical characterization and purification of human stem/progenitor cells has proceeded along lines which are parallel to the mouse system. Indeed, because of clinical impetus, it can be argued that they are further advanced. Thus, as measured in the range of assays discussed above, the consensus physical phenotype of the most primitive portion of the human stem/progenitor hierarchy is CD34+Lin-CD38- (Terstappen, L.W.M.M., et al. 1991). The CD34+Lin-CD38+ subset contains less primitive, more committed cellular entities. Other studies have shown that, similar to the mouse, low level expression of Thy1 (CD90) is a feature of the primitive human stem cell (CD34+Lin-CD90+)(Baum, C.M., et al. 1992; Craig, W., et al. 1993). Most CD90+ cells in this compartment are CD38-. Therefore, the consensus phenotype can be described as CD34+Lin-CD90+ (Craig, W., et al. 1993). Two potential differences with the murine system can now be highlighted. First, a very recent and elegant study has shown that the most primitive mouse stem cell may in fact be CD34-/lo (Osawa, M., et al. 1996) . Whether this is a genuine difference or whether it reflects the ability to perform more accurate long-term engraftment studies in the mouse remains to be determined. Second, it has been suggested that in the mouse, CD38 expression is a positive indicator for primitive stem cell function in a purified population (Randall, T.D., et al. 1996) . As in the mouse, human stem/progenitor cells have been identified and purified from various sources. These include: adult BM (Baum, C.M., et al. 1992), CB (DiGiusto, D.L., et al 1996) , fetal liver (Craig, W., et al. 1993) and peripheral blood stem cells after various mobilization protocols (Murray, L., et al. 1994). Similar to the data obtained in the murine system, comparative studies reveal that, in general, the basic and fundamental functional properties of stem/progenitor cells are shared regardless of the tissue source. There are however, significant functional and physical differences.

Numerous insights into hematopoictic molecular control mechanisms have come from gene-targeting studies in mice. Mutations in specific genes, most notably, those encoding transcription or DNA-binding proteins, have profound cell-intrinsic, global or lineage-specific effects on hematopoietic development (Shivdasani, R.A., and Orkin, S.H. 1996; Orkin, S.H. 1996). In the latter cases, it is tempting to speculate that the phenotypes result from defects in the commitment process. However, malfunctions in the commitment decision to "set up" a program of differentiation are difficult to distinguish from malfunctions in the differentiation program itself. Two gene products, AML1 (CBF2) and SCL (tal-1) appear to be necessary global regulators of hematopoiesis (Wang, W., et al. 1996; Okuda, T., et al. 1996; Porcher, C., et al. 1996; Robb, L. , et al. 1996) . Whether these molecules act to specify a hematopoietic stem cell or by other means is an open question. Interestingly, both of these molecules play roles in leukemic transformation. A very important gain of function study documents the apparent ability of HOXB4 to increase primitive cell numbers without significant impairment of differentiation abilities (Sauvageau, G., et al. 1995) . Together with observations that HOXA9 is translocated in myeloid leukemia (Nakamura, T., et al. 1996, Borrow, J., et al. 1996) , these studies suggest an important hematopoietic role for homeobox proteins. Without question, the above and other studies have identified important regulators of hematopoiesis. However, in almost all cases these regulators were first identified in other systems. The opposite approach is to directly search for stem cell regulators in stem cells, Graf, L., and Torok-Storb, B. 1995; Yang, Y., et al. 1996). The present invention solves these problems.

SUMMARY OF THE INVENTION

The human hematopoietic stem/progenitor cell population has been extensively characterized according to physical and antigenic criteria as well as in a variety of in vitro and in vivo assay systems. Collectively the human studies have revealed similarities to the hierarchical stem/progenitor cell organization defined in the murine system. In spite of significant strides in the identification of cytokines which can act on stem cells, it has not been possible to define a system where undifferentiated expansion of the most primitive stem cell population occurs. Similarly, it has not been possible to direct differentiation along lineage-specific pathways. These limitations, which also apply to the murine system, have hampered the elucidation of regulatory mechanisms which

mediate the most fundamental aspect of stem cell biology; that is, the decision to self-renew or commit to differentiation. As a consequence, very little is known about the molecular biology of the most primitive hematopoietic stem cell in any organism. It was hypothesized that the regulation of primitive stem cells will be mediated at least in part by the products of genes which are uniquely or predominantly expressed in these cells. One precedent for an important, differentially-expressed molecule is the flk2/flt3 receptor tyrosine kinase. There presumably are other important and differentially expressed gene products. Therefore, it is an object of the present invention to identify these molecules and address their functional roles. Specifically, an aspect of identification of gene expression patterns specific to primitive human stem cells is the molecular phenotype of the human stem cell. The present invention describes methods to define the profile of genes specifically expressed in undifferentiated human stem/progenitor cell populations.

A primary focus of the present invention is on primitive cells isolated from normal bone marrow (BM) samples. The present invention further comprehends use of other sources of stem cells, such as umbilical cord blood (CB).

The methods of the present invention combine diverse technical approaches and sophisticated bioinformatic analyses.

This invention further provides methods to identify genes whose expression can be modulated by cytokine or stromal-dependent culture and/or by cell-cycle status.

Another object of the present invention is to provide methods for the functional characterization of human stem cell-specific gene products. An aspect of this invention is a method to facilitate the functional characterization of specifically expressed gene products as candidate regulators of a variety of stem/progenitor cell processes. In particular, a provided method uses an in vitro system which approximates many characteristic properties of normal stem cells to analyze positive and negative regulation of proliferation, cell-cycle parameters, apoptosis and commitment.

It is a further goal of the present invention to provide a necessary (and usually missing) component for stem cell gene-expression screens; that is, the ability to quickly assess the function of extensive panels of genes.

It is also an object of the present invention to provide a method for the functional identification of stem cell regulators. An aspect of this invention is a facile screening method for "categorizing" large populations of specifically-expressed molecules according to their potential roles in a variety of stem/progenitor cell processes. Gain of